

“An Enquiry into the Nature of the Vesicating Constituent of Croton Oil.” By WYNDHAM R. DUNSTAN, M.A., F.R.S., and LUCY EVEREST BOOLE, F.I.C., Lecturer on Chemistry in the London School of Medicine for Women. Received June 5, —Read June 20, 1895.

The nature of the vesicating, or more strictly the pustule-producing, constituent of croton oil is a long out-standing problem in chemical pharmacology. Many attempts have been made to disentangle from the complicated mixture of fatty acids and glycerides expressed from the seeds of *Croton tiglium*, known as croton oil, a single substance exhibiting its remarkable power of raising pustules on the skin. The conclusion first arrived at by Pelletier and Caventon that the vesicating constituent is a volatile acid was afterwards proved to be incorrect. The early experiments of Nimmo and of Warrington showed that alcohol is able approximately to separate the oil into two parts, that which dissolves containing nearly the whole of the vesicating constituent. The systematic investigation, of which an extensive and somewhat involved account was published by Buchheim* in the year 1857, confirmed and extended these observations. It was then shown that the small proportion of croton oil which may be dissolved by repeated extraction with alcohol (85 per cent.) contains nearly the whole of the vesicating constituent. But the bland oil which remains undissolved still retains the purgative power of the original substance. When the solution of the vesicating portion is mixed with aqueous ammonia and lead oxy-acetate, the vesicating constituent remains in solution, and the precipitate contains only the lead salts of fatty acids. The vesicating oil may be saponified by alkalis the resulting soap still retaining the vesicating property which is also exhibited by the magnesium, barium and lead salts prepared from the soap. A partial purification of the vesicating substance may be effected by extracting the lead salt with ether which dissolves an inactive lead salt (lead oleate), but leaves a lead salt possessing powerful vesicating properties and furnishing, when decomposed with hydrochloric acid, an oily acid exhibiting the same property. The ethyl salt, prepared in the usual way from this acid, is however not vesicating, neither is the acid regenerated from it. From these results Buchheim concluded that the vesicating property of croton oil is due either to a peculiar non-volatile acid which he named crotonoleic acid, or perhaps to a hydrolytic product of this acid. On the other hand he suggests that the vesicating property may belong

* Virchow's 'Archiv,' vol. 12, p. 1; see also his 'Lehrbuch der Arzneimittellehre,' p. 364.

to some other constituent which is present as an impurity in this acid, and cannot be separated from it. In this paper Buchheim does not decide in favour of either of these hypotheses, but on the whole leans towards the last supposition. A year later, the subject was investigated by Schlippe,* who was apparently not familiar with Buchheim's observations. Schlippe showed that the vesicating constituent, obtained by solution of the croton oil in strong alcohol, was destroyed by heating it with alkalis. He concluded that it is not an acid, but a polyhydric alcohol of the formula $C_{18}H_{14}O_4$; and he proposed for it the name of *crotonol*. In 1873 Buchheim† published an account of further work on the vesicating constituent. Repeating the experiments of Schlippe, he came to the conclusion that this observer was mistaken in considering crotonol as the active constituent of the oil. Buchheim maintains that his new experiments have conclusively proved that the vesicating principle is an acid, *crotonoleic acid*. This substance he isolated in the following manner: croton oil was shaken several times with alcoholic ammonia, the upper alcoholic layer, which contains the vesicating constituent, being each time removed from the insoluble oil below. The alcoholic solution was mixed with an aqueous solution of barium chloride, and warmed. The barium salt thus formed was filtered off, washed and dried. It was then repeatedly extracted with ether, which dissolves the barium salt of oleic and crotonoleic acids, but leaves the barium salts of other fatty acids (stearic, palmitic, &c.) undissolved. The ethereal solution of the barium salts is decomposed by shaking it with dilute hydrochloric acid. The ether, having been washed with water, is distilled and the oleic and crotonoleic acids are then left in the form of an oil. This oil is dissolved in alcoholic ammonia, and the liquid fractionally precipitated with a solution of barium chloride, the last precipitate being subjected to a repetition of the process until finally a barium salt having a constant composition is obtained. By this means the less soluble barium oleate may be separated from the more soluble barium crotonoleate. The acid regenerated from the barium salt is an oil, which Buchheim considered to be the same as Schlippe's crotonol.

Crotonoleic acid is slowly decomposed when heated with alkalis or strong acids, losing its vesicating property. Buchheim did not prepare the acid pure enough to admit of ultimate analysis, but from its behaviour he concludes that crotonoleic acid is closely related to the ricinoleic acid of castor oil. Its glyceride does not vesicate; and, according to Buchheim, croton oil contains the glyceride as well as the free acid, so that, if the bland oil remaining after the removal

* 'Annalen der Chemie und Pharmacie,' 1858, vol. 105, p. 1.

† 'Archiv der Heilkunde,' 1873, vol. 14, p. 4.

of the crotonoleic acid is saponified, the vesicating property is found in the acids regenerated from the soap.

In 1883 H. Senier,* apparently unaware of previous researches on the subject, including those of Buchheim, published an account of observations which led him to conclusions similar in some respects to those previously arrived at by Buchheim. Senier found that strong alcohol separates croton oil into two parts: a small part which dissolves, carrying with it the whole of the vesicating constituent, and the main part which does not dissolve, but retains the purgative power, although it has lost the vesicating property. The vesicating constituent is not a free acid since it is not removed from croton oil by agitation with aqueous sodium carbonate. When the soluble oil is saponified with potash the vesicating property is no longer exhibited by the soap; but the acid regenerated from the soap vesicates powerfully. By a process of fractional saponification, followed by fractional precipitation of the fatty acids, Senier concluded that the vesicating constituent is a non-volatile fatty acid, closely resembling oleic acid. The glyceride of this acid is less readily saponifiable than the other glycerides present in croton oil; and, similarly, the acid is regenerated first when the soaps are decomposed, being thrown out with the acids of lowest melting point.

In 1890, the question as to the nature of the vesicating constituent was reinvestigated by Hirscheydt,† working in Professor Kobert's laboratory at Dorpat. It is pointed out, in his voluminous paper, that alcohol fails to effect a sharp separation of the vesicating from the non-vesicating portion of croton oil; that different specimens vary in their solubility in alcohol; and that, although the vesicating constituent is far more soluble, yet, if sufficient alcohol be used, the whole of the oil will gradually pass into solution. A repetition of Buchheim's experiments confirmed his conclusion that a non-volatile fatty acid is the cause of the vesicating property; and for this acid it was proposed to retain the name of "crotonoleic acid," originally suggested by Buchheim. By purifying the barium salt, the acid was ultimately obtained in a purer condition than it had been produced by Buchheim. It was not submitted to ultimate analysis; but since its barium salt, prepared in the pure state by fractionally precipitating the sodium salt with barium chloride, contained less barium than barium oleate, it is judged to have a higher molecular weight than oleic acid. The acid is a brown oil at the ordinary temperature, and is very unstable, especially when in contact with alkalis, which, if heated with it, slowly destroy its vesicating power.

The following improved process for the isolation of crotonoleic acid

* 'Pharm. Journ.,' III, vol. 13, p. 446.

† 'Dorpat Arbeiten,' 1890, vol. 4, p. 5.

is suggested by Hirscheydt:—The part of the croton oil which is readily soluble in strong alcohol is heated on the water bath with a hot saturated solution of barium hydroxide, until saponification is complete. The barium soap thus formed is well washed with water, until the soluble barium salts (acetate, butyrate, tiglate, &c.) are removed, and it is then dried by warming it in a vacuum. The dried mass is next repeatedly extracted with ether, which dissolves the barium salts of oleic and crotonoleic acids, but not those of palmitic, stearic and other solid fatty acids.

In order to separate the barium crotonoleate from the barium oleate which accompanies it in the residue left on the evaporation of ethereal solution, the mixture is extracted with cold absolute alcohol, which dissolves only the crotonoleate. The barium crotonoleate thus obtained is decomposed by the addition of dilute sulphuric acid, and the crotonoleic acid separated by extracting the mixture with ether in the usual manner.

By this means Hirscheydt claims to have produced crotonoleic acid in a purer and more active condition than that in which it was obtained by Buchheim. He points out, however, that the new process is not a wholly satisfactory one for the production of the acid, since some is inevitably decomposed by the barium hydroxide, even if every precaution is taken to avoid over-heating the mixture during saponification.

Crotonoleic acid thus prepared has been employed medicinally, and is now an article of commerce.

It will be seen from the foregoing brief sketch of previous experiments on the nature of the vesicating constituent of croton oil that, although conflicting accounts have been given, the evidence which has been so far accumulated goes to prove that the active substance is the glyceride of an acid to which the name of crotonoleic acid is given, the acid, as well as its salts, having vesicating properties. It is, however, very doubtful, from the nature of the processes which have been employed to isolate the compound, and from the description of its properties, whether it is a single substance. It appeared to us important to attempt to obtain the pure compound and then to ascertain precisely its chemical constitution, so that we might be able to understand the kind of structure which is associated with the vesicating property, especially since no other fatty acid of known constitution exhibits this peculiarity.

Examination of Crotonoleic Acid.

The crotonoleic acid used in these experiments was either prepared by ourselves from croton oil, following the Dorpat directions given above, or it was procured from Merck of Darmstadt, who prepares

it for medical use, strictly according to this same process. It is a thick oil, possessing very powerful vesicating properties. In the first instance the substance was distilled with steam but nothing passed over, and the oil was apparently unchanged. Distillation under the ordinary pressure produced entire decomposition. Distillation under reduced pressure gave no better results. A liquid began to distil near 200°C . (50 mm.), but this was evidently a product of decomposition, the quantity of which increased as the temperature rose gradually to 250°C . None of the fractions exhibited vesicating power, and the residue was also inactive.

Fractional precipitation of the lead salt of the acid was next adopted, in order to ascertain whether the substance was homogeneous. A solution of lead acetate in alcohol was added to an alcoholic solution of the crotonoleic acid. Water was then mixed with the clear solution, until a permanent turbidity resulted. The oily lead salt which soon settled was removed and decomposed with dilute hydrochloric acid, and then the free crotonoleic acid was extracted with ether. The alcoholic mother liquor was precipitated a second time with lead acetate and water, and the acid similarly regenerated from the lead salt. Since a third fraction could not be obtained from the alcoholic solution, this was decomposed and the acid recovered from the dissolved lead salt. Since our object was to ascertain whether the original material was homogeneous and consisted entirely of the vesicating constituent of croton oil, and as there is no chemical test for the presence of the active substance, we were obliged to fall back on a physiological method of detecting it and indicating approximately the extent to which the active substance was contained in different specimens of material. The plan adopted was to dissolve equal weights of the substances in the same weight of oleic acid, and then to apply a single drop of each solution to the arm; the extent of the blistering which ensued being carefully noted. This we have found to be the only method readily available for gauging approximately the vesicating power of different specimens. When the three specimens, fractionated from the original crotonoleic acid, were tested in this manner, the first was found to be quite inactive, the second to be nearly inactive, whilst the third and smallest fraction proved to be a violent vesicant, far exceeding in power the original crotonoleic acid. A number of these fractionation experiments were performed, always with the same result, thus conclusively proving that crotonoleic acid is a mixture for the most part composed of non-vesicating material. When the original substance is fractionally precipitated with lead acetate the non-vesicating constituents are precipitated first, leaving nearly all the active material in solution. It therefore seemed probable that by taking advantage of this difference in the solubility of the lead salt in dilute alcohol we might be able to isolate

the pure active substance. Starting with crotonoleic acid, and with the last fraction of the first operation, repeating several times the process of fractional precipitation (assisting the subsidence by the addition of kaolin), a substance was obtained the vesicating power of which did not seem to be increased by further treatment. The quantity produced from the crotonoleic acid was, however, comparatively small, and, as the process of obtaining the "acid" itself from croton oil is extremely tedious and unproductive, we decided to seek for some other method of isolating the active substance, it being no longer of any importance to work with crotonoleic acid, since we had conclusively proved this substance to be a mixture.

Isolation of the Vesicating Constituent from Croton Oil.

To begin with, an attempt was made to prepare a mixed lead salt by precipitation from the sodium salts resulting from the saponification of croton oil with soda. The croton oil dissolved in alcohol was boiled for some hours with alcoholic soda. The soap thus prepared was found to be non-vesicating, and so also were the fatty acids regenerated from it. It was therefore certain that the vesicating constituent of croton oil is decomposed by heating with alkalis, a conclusion which has since been substantiated by numerous experiments.

For this reason the lead salts were prepared by direct saponification of croton oil with a mixture of lead oxide and water. The lead "soap" thus produced was a yellowish-white sticky mass, possessing apparently all the vesicating power of the original oil. After having been thoroughly washed with warm water, the soap was dried in the water bath, mixed with sand, and the powdered mixture digested with ether in a stoppered bottle for about four weeks. The ether was then poured off, and the digestion repeated during one week with two successive quantities of ether. The ethereal solutions were mixed together and distilled, when a thick brown sticky mass remained; and this seemed to be considerably more vesicating than the original lead soap; the residue of lead salt insoluble in ether was much less active than the original mixture, whilst by repeated extraction of a part of it with ether it became almost devoid of vesicating property. It is thus clear that the vesicating substance passes into solution in ether together with lead oleate. The ethereal residue was now extracted several times with strong alcohol, and, although only a small proportion dissolved on each occasion, the material became less and less vesicating and finally quite inactive. By this means the greater part of the lead oleate was separated, whilst the whole of the vesicating substance was dissolved by the alcohol. The alcoholic solution left on distillation a brown, nearly

solid substance, having extraordinary power as a vesicant. Alkalis, even in the cold, slowly decomposed it, the vesicating property being gradually lost. The acid recovered from this material, which was presumably a lead salt, proved to be highly active. During this regeneration we were surprised to notice that the material contained very little lead, so little as to render it almost impossible for the vesicating substance to be itself a salt of lead. In order to investigate this important question, some of the material was dissolved in alcohol and precipitated with a solution of sodium carbonate. The lead carbonate, which was but little, having been filtered off and the alcohol removed by boiling the liquid, the solution of sodium salts was extracted with ether. This dissolved a small quantity of a yellow oil, which nearly solidified on standing, forming a transparent resinous substance. This was proved to be more active as a vesicant than any substance hitherto prepared. It burned without ash and was therefore not a sodium salt. The solution which had been extracted with ether contained "soap," which was inactive, as were also the fatty acids regenerated from it. Similarly, if an alcoholic solution of the sodium salts (prepared from the lead salts) is precipitated with silver nitrate, the silver salt is found to be inactive; but if the filtrate is extracted with ether a powerfully vesicating resinous substance is removed *which contains no silver*; and it was thus ascertained that the vesicating constituent of croton oil is a resinous substance which is not an acid and cannot be saponified by a mixture of lead oxide and water. This substance may be provisionally named "croton-resin."

Isolation of Croton-resin from Crotonoleic Acid.

It now became of interest to show that crotonoleic acid, the supposed active constituent of croton oil, owes its activity to the croton resin it contains, and not to the "acid" which is present. Some quantity of crotonoleic acid was heated with a mixture of lead oxide and water. The resulting lead soap was thoroughly washed with water and extracted with alcohol, which dissolved about one-half. The alcoholic solution was mixed with aqueous sodium carbonate, the alcohol dissipated, and the aqueous solution extracted with ether until it became virtually inactive. The ethereal solution left, on evaporation, the croton resin which we had previously obtained from croton oil. The inactive aqueous soap solution, when decomposed, furnished a mixture of fatty acids, which was quite inactive.

By a different method of procedure the same result was obtained. Crotonoleic acid was warmed with aqueous sodium carbonate, which left a portion, chiefly croton resin, undissolved. As soon as action was over, the liquid was extracted with ether, which removed the

croton resin, leaving an inactive solution of sodium salts. The resin thus obtained was purified by dissolving it in alcohol and fractionally precipitating the solution with water. The last fraction was extremely active and when dry become a pale yellow, brittle, resinous mass devoid of acid properties. It was therefore conclusively proved that the so-called crotonoleic acid, hitherto regarded as the active constituent of croton oil, is a mixture of fatty acids, chiefly oleic acid, with croton-resin, which is the true vesicating constituent of croton oil.

Preparation, Properties, and Composition of Croton-resin.

The method by which the resin was first isolated has already been described. It was found advantageous to employ dilute alcohol in extracting the resin from the lead soap, since in this case very little lead salt was dissolved and the alcoholic solution needed but little fractional precipitation with water in order to obtain a pure product.

Inasmuch as it is known that alcohol dissolves the vesicating constituent from croton oil, leaving a bland oil behind, it seemed probable that the resin might be isolated by means of fractional precipitation from this alcoholic solution of croton oil, and the process of saponification altogether avoided. When freshly expressed croton oil is shaken with rather more than twice its volume of alcohol (50 per cent.), very little dissolves. After promoting subsidence by the addition of kaolin or asbestos, the mixture was filtered, and the alcoholic solution evaporated to dryness, when a thick oil remained. This process was thrice repeated on the original croton oil. The residues were dissolved in alcohol, and the solution precipitated in a number of small fractions by the addition of water. The first fractions were dark in colour and oily in consistence, whereas the last fractions were pale yellow, and solidified to pale yellow resins which were highly active. In this process of fractional precipitation, crystals separated in the early stages; these were proved to be cholesterol, which is evidently present in some quantity in croton oil. A still easier method of separating pure croton resin from croton oil consists in extracting the oil with more dilute alcohol (25 per cent.), when the resin is dissolved in a nearly pure state. The yield is, however, so small that the method is not well adapted for obtaining any quantity of the substance. The most satisfactory process for separating croton-resin from croton oil which we have so far found is the following, which consists in removing the resin as far as possible from the oil by digesting it with alcohol, then converting the glycerides in this mixture into a lead soap, from which the resin is extracted by alcohol and purified by fractional precipitation with water. Croton oil is warmed on the water bath for some hours with about twice its volume

of alcohol (70 per cent.). The oil is allowed to settle, and the alcohol decanted. The residual oil is again twice extracted with alcohol in the same manner. The mixed alcoholic solutions are then distilled to a small volume and the oil saponified by heating it on the water bath with a mixture of freshly precipitated lead oxide and water. The lead soap is thoroughly washed with warm water, mixed with white sand, dried, powdered, and percolated with warm alcohol. Dilute alcohol (25 per cent.) removes the resin almost pure; but the entire extraction of the active constituent takes a long time, and we have found it in the end shorter to extract with strong alcohol (70 per cent.), which dissolves also a little lead oleate, and then to purify the resin by fractional precipitation of the alcoholic solution with water. The first fraction usually contains the whole of the lead salt, and the later fractions furnish a pale yellow, hard, brittle resin, which is generally quite pure, or needs but one repetition of the process of fractional precipitation to make it so. Croton-resin is a pale yellow, brittle, nearly transparent substance, which is easily reduced to a fine powder. Numerous attempts have been made in various ways to crystallise it, or to separate some crystalline substance from it, but in every case without success. It contains carbon, hydrogen, and oxygen, but no nitrogen. Its homogeneity and composition have been established by the analyses of various specimens representing different fractions obtained by the addition of small quantities of water to its alcoholic solution. A specimen of the resin, which had been fractionated in the manner described, was dissolved in dry ether, and the ethereal solution evaporated in a large flat-bottomed dish. The thin film thus formed was heated for two hours in the water bath and then exposed for some weeks in a vacuum desiccator over sulphuric acid. A portion of this specimen (A) was burned in two parts, with the following results:—

I.	Weight of resin	0·2009
	Weight of water	0·1496
	Weight of carbon dioxide ..	0·4902

C = 66·55 per cent.; H = 8·26 per cent.

II.	Weight of resin	0·18
	Weight of water	0·1296
	Weight of carbon dioxide ..	0·438

C = 66·4 per cent.; H = 8 per cent.

Mean for A—

C = 66·45 per cent; H = 8·13 per cent.

The remainder of the specimen was dissolved in alcohol, and the

solution precipitated by water in three distinct fractions (A_1 , A_2 , A_3), the first (A_1) and the last (A_3) of which were burned.

A_1	Weight of resin	0.1648
	Weight of carbon dioxide..	0.4028
	Weight of water.....	0.126

C = 66.6 per cent.; H = 8.49 per cent.

A_3 (1).	Weight of resin	0.1992
	Weight of carbon dioxide..	0.4736
	Weight of water.....	0.4424

C = 64.9 per cent.; H = 7.94 per cent.

A_3 (2).	Weight of resin	0.1974
	Weight of carbon dioxide..	0.474
	Weight of water.....	0.1458

C = 65.4 per cent.; H = 8.18 per cent.

Mean for A_3 (1 and 2) C = 65.15 per cent.; H = 8.02 per cent.

These results show that the original resin (A) was almost pure, since the composition of the first fraction (A_1) corresponds with that of the original (A), whilst between (A) and the last fraction (A_3) is a mean difference of only 1.3 per cent. in the carbon, and 0.1 per cent. in the hydrogen. Combustion of different fractions furnishes the most delicate, and in this case the only, available test of homogeneity. The action of all three fractions on the skin was exceedingly severe, and it was not possible to distinguish between them. In order to gain conclusive evidence of the constancy of the composition of croton-resin, a fresh quantity of croton oil was dealt with in the manner previously described, and the resin purified by fractional precipitation until it was obtained as a pale yellow hard solid. The new specimen (B) on combustion furnished the following data :—

I. C = 64.67 per cent.; H = 7.94 per cent.

II. C = 64.6 per cent.; H = 7.8 per cent.

Mean for B—

C = 64.63 per cent.; H = 7.87 per cent.

These results agree as well as could be expected with such a material with the mean of those obtained with fraction (A_3) in the previous experiment; and, taking the mean of these four combustions of the two different specimens, we get

C = 64.89 per cent.; H = 7.94 per cent.

which agree best with the formula $C_{13}H_{18}O_4$, the calculated percentages being

$$C = 65.54 \text{ per cent. ; } H = 7.56 \text{ per cent.}$$

Croton-resin is nearly insoluble in water, light petroleum, and benzene, but dissolves readily in alcohol, ether, chloroform, acetic ether, and amyl alcohol; and is deposited, even by the very slow evaporation of these solvents, as a pale yellow resin. Its solution produces no effect on polarised light. The resin has no fixed melting point. A few degrees above the atmospheric temperature it softens and very gradually melts as the temperature is raised being quite fluid at $90^{\circ} C$.

Croton-resin has neither basic, nor acidic properties. It is insoluble in dilute acids; and nearly so in solution of sodium carbonate from which it can be extracted unchanged by shaking with ether. It does not act with metallic oxides and may be boiled with a mixture of water and lead oxide without being appreciably affected. On the other hand it is very slowly decomposed and dissolved by contact with aqueous caustic alkalis and is very rapidly changed if heated with them, the resin being gradually dissolved with much darkening in colour and entire loss of its vesicating power. Ammonia acts much less rapidly. Water, heated with the resin in a closed tube at $200^{\circ} C$., slowly decomposes it, with formation of a dark coloured resin and a mixture of acids. Dilute acids, also, slowly decompose the resin when heated with it in a closed tube at $200\text{--}250^{\circ} C$.; much darkening in colour takes place and several acids are formed, but they have not hitherto been obtained in sufficient quantity to separate and identify. One would appear to be valerianic acid. As the total quantity of pure material at our disposal has been very small, it has not been possible, so far, to do much towards elucidating the question as to the constitution of croton-resin. This seems to be complicated, since on decomposition with alkalis it furnishes a mixture of acids, some of which seem to be higher members of the acetic series. When the resin is heated with strong potash solution (50 per cent.), it is quickly decomposed, a black resinous mass being produced in addition to "soap," from which a mixture of fatty acids was liberated. A very small quantity of one of these was separated in a nearly pure state as an oil at the ordinary temperature. It appeared to be oleic acid, since its silver salt contained 26.38 per cent. of silver, whilst silver oleate contains 27 per cent. When the resin is heated with a 10 per cent. solution of soda in a closed tube at $100^{\circ} C$. it completely dissolves. As is usually the case, when the resin undergoes hydrolysis, a dark coloured quite inactive resin, insoluble in ether, is produced, together with a mixture of acids. When the solution is acidified and distilled, a small quantity of a

“fatty” acid passes over, and a further quantity may be obtained by extracting the residue in the flask with ether. The acid was neutralised with ammonia, and the aqueous solution precipitated with calcium chloride. The semi-crystalline precipitate was dissolved in alcohol and crystallised from this solution. The acid removed from the calcium salt was neutralised with ammonia, and the aqueous solution of the ammonium salt precipitated by silver nitrate. The white voluminous precipitate was dried, and the silver estimated. Another portion of the precipitate was dissolved in boiling alcohol, and the crystals that separated from the solution were also analysed, with the object of determining whether the silver salt was a single substance or a mixture. The percentage of silver in the original silver salt was 30·5, whilst that in the salt crystallised from alcohol was 31·1. The acid, regenerated from this silver salt, crystallised from dilute alcohol, and melted at a low temperature. In order to further characterise this acid a fresh quantity of the silver salt was crystallised and burned with the following results :—

Weight of salt.....	0·1169
Weight of water.....	0·0831
Weight of carbon dioxide ..	0·212
Weight of silver	0·0360

C = 49·4 per cent.

H = 7·89 „

Ag = 30·79 „

These results indicate that the acid is one of the higher members of the acetic series, probably either palmitic or myristic acid. The percentages of silver, calculated from the formulæ of the silver salts of these acids, are, for silver palmitate, 30 per cent., and silver myristate, 32 per cent. The percentage of carbon found is however too low for palmitic acid, but approximates to that contained in myristic acid. The question as to which member of the series the acid corresponds with can however only be decided by further experiments with larger quantities than we were able to command. Although a fatty acid is produced when alkalis act on the resin, we could find no evidence of the production of glycerine. It may be concluded, therefore, that the resin is not a glyceride, as was probable from the mode in which it was isolated. When warmed with strong nitric acid croton resin undergoes oxidation without forming any nitro-compound. A number of acids are produced in this change of which only a preliminary examination has been made. There are at least two fatty acids formed, one of which closely resembles palmitic acid, as well as valerianic acid and oxalic acid.

The hydrolytic and oxidation products of the resin make it quite

clear that the molecule is a very complicated one, and that the empirical formula deduced from the analytical data must be doubled at least and written $C_{26}H_{36}O_8$. With regard to the group of organic compounds in which croton-resin should be included, the examination of its properties points to the conclusion, to be substantiated by further work, that, in all probability, it possesses the structure of lactone or anhydride. It has already been shown that it has neither basic nor distinctly acidic properties. It does not react with bisulphites or with phenylhydrazine, nor does it reduce Fehling's solution. It is therefore not a ketone or an aldehyde. Attempts to form compounds or derivatives of it by the action of various agents have not been successful. It may be well to record here that auric chloride, silver nitrate, mercuric chloride, lead acetate, cadmium iodide, iodine, and picric acid do not react with an alcoholic solution of croton-resin.

The task of unravelling the constitution of this substance would be a very difficult one if not an impossible one with our present knowledge, and the difficulty is greatly increased by the circumstance that it is apparently incapable of being crystallised. The amount of croton resin present in croton oil is small; probably it does not exceed 3 per cent. The extraction of the resin is not only a very tedious and troublesome operation, but it is one not unattended with danger to health. It attacks the mucous membrane and the skin with great severity; and we have evidence that when the body is continually exposed to its action on one part or another—by contact with the hand, or by the access of minute particles to the nose and throat, as may occur during manipulation of the dry substance, or during the evaporation of solutions containing it—the resin produces a series of secondary effects which seem worthy of special study.

It will be seen that our experiments have led us to a conclusion opposed to that drawn by Buchheim, and more recently by Kobert and Hirscheidt. We consider that we have disproved their conclusion that the so-called "crotonoleic acid" is the vesicating constituent of croton oil. Of previous workers Schlippe had obtained results most in accordance with our own. He correctly suggested that the vesicating constituent is not an acid; and it is probable that the "crotonol" obtained by this chemist chiefly consisted of croton-resin.

In concluding this account of our work, the expenses of which have been defrayed by a grant from the Royal Society out of the Government Fund, we wish to express our obligations to the well-known firm of Messrs. Horner and Sons, of Aldgate, for having furnished us with freshly expressed oil of undoubted purity. The inquiry has been conducted in the Research Laboratory of the Pharmaceutical Society.